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ANTIMICROBIAL ENFORCEMENT ANALYTICAL METHOD VALIDATION

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INTRODUCTION

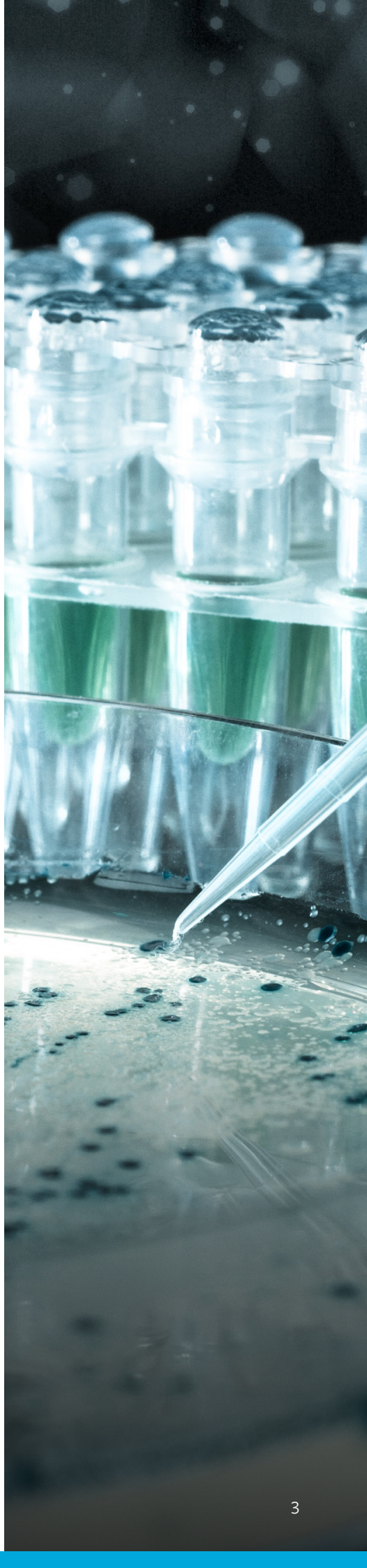
The Importance of Analytical Method Validation

When validating analytical methods for Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), it is important to understand the guidelines and their specific requirements.

The objective of validation of an analytical procedure, regardless of the type of procedure, is to demonstrate that it is suitable for its intended purpose.

Under FIFRA, antimicrobial products designed to protect public health in the United States must be registered with the United States Environmental Protection Agency (EPA). To support these registrations, the EPA requires registrants to provide toxicology data, antimicrobial efficacy data and product chemistry data designed to support the purported label claims. As part of the product chemistry data, the EPA's Office of Chemical Safety and Pollution Prevention (OCSPP) requires registrants to provide an analytical method suitable for enforcement purposes for each active ingredient in the product and for each other ingredient or impurity that is determined to be toxicologically significant. An exhaustive validation of the analytical method using the formulated product provides the highest level of confidence that the method is sound. This article is designed to summarize the key considerations for a thorough analytical method validation.

When validating an analytical method, the recommended parameters that should be considered include, but may not be limited to, precision, accuracy, specificity, linearity, quantitation limit, detection limit, robustness and system suitability.



8 PARAMETERS TO CONSIDER

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PRECISION

The precision of an analytical method expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogenous sample under the same prescribed conditions. The precision of a method is critical to ensuring similar results can be consistently achieved. To validate the precision of an analytical method, a series of sample replicates of the formulated product is prepared, and each sample is individually analyzed for percent active concentration. A percent relative standard deviation (%RSD) is calculated from the sample set and is compared to an accepted %RSD value to validate the precision of the method for the specific product.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

- **Repeatability** expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.
- **Intermediate precision** expresses within-laboratories variations: different days, different analysts, different equipment, etc.
- **Reproducibility** expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

The precision of a method is critical to ensuring similar results can be consistently achieved.

ACCURACY

The accuracy of an analytical method expresses the closeness of agreement between the value found and the value that is accepted either as a conventional true value or other accepted reference value. The accuracy of a method is critical to ensuring the results of the method are true. A typical method for validating the accuracy of an analytical method is to spike a placebo formulation (identical product formulation with no active ingredient) with known low, mid and high levels of active ingredient. The concentrations of low, mid and high levels, should, at minimum, cover the potential range of active ingredient concentrations in the final manufactured product (lower to upper control limit). The prepared solutions are analyzed for percent active concentration and a percent recovery value is determined for each level of active by comparing the "found" concentration to the "theoretical" concentration. The percent recovery is then compared to an accepted value to validate the accuracy of the method for the specific product.

SPECIFICITY

Specificity is the ability to unequivocally assess the active ingredient or compound of interest (analyte) in the presence of components that are expected to be present in the product (e.g. inert ingredients, impurities or other product matrix components). Specificity of an analytical method is critical to confirm that the non-target components of the product are not biasing the test results. To validate the specificity of a method, a placebo formulation can be analyzed using the analytical method to screen for interferences associated with non-target compounds. The level of interference (whether determined by instrumental methods such as liquid chromatography or by manual titration) can be confirmed to be below accepted levels to validate the specificity of the method for the target compound within the formulated product.

This definition has the following implications:

- **Identification**: to ensure the identity of an analyte.
- **Purity**: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.
- **Assay**: to provide an exact result that allows an accurate statement on the content of the analyte in a sample.

LINEARITY

The linearity of an analytical method is its ability (within a given range) to obtain results that are directly proportional to the concentration of the target analyte in the sample. The linearity of a method is an important component to calculating unknown concentrations based off known standard values. The relationship must be linear to draw accurate conclusions.

To validate the linearity of an analytical method, a standard solution is commonly prepared using a series of concentrations bracketing the expected concentration of the analyte. The solutions are analyzed according to the analytical method and the measured results are plotted against the "theoretical" concentrations. Linear regression analysis is performed on the plotted data and the resulting R^2 value from the line-of-best-fit is compared to an accepted value to validate the linearity of the method with a given range.

QUANTITATION LIMIT

The quantitation limit of an analytical method is defined as the lowest amount of analyte in a product that can be reliably quantified. This criterion is especially important when analyzing very low-level analytes in order to establish the quantifiable limit of the analytical method. The quantitation limit is directly related to the readability of the burette being used for titration-based methods or to the signal-to-noise ratio of a diluted product solution in instrumental methods such as high performance liquid chromatography (HPLC) or gas chromatography (GC).

DETECTION LIMIT

The detection limit of an analytical method is defined as the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated. This aspect of a validation is typically not required except in the case of very low-level active ingredient analysis or impurity detection methods where simply detecting the presence of an impurity might be just as important as determining its concentration. For instrumental methods, the detection limit is directly related to the signal-to-noise ratio of a diluted product solution.

ROBUSTNESS

Robustness is the measure of an analytical method's capacity to remain unaffected by small, but deliberate, variations in procedural parameters and provides an indication of the method's reliability during normal or typical execution. Common primarily to instrumental methods, the following robustness conditions can be considered:

- Mobile phase pH ranges
- Mobile phase concentration ranges
- Ratio of solvent variations
- Column lot variations
- Column temperature ranges
- Flow rate ranges
- Injection volume ranges

SYSTEM SUITABILITY

System suitability validation tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability parameters for instrumental methods are measures of the limits, within which the procedure can be defined as “normal operation.” Potential parameters include, but are not limited to:

- System suitability (system precision)
- Check standard recovery (system drift)
- Resolution (in the case of more than one peak)
- Theoretical plate count
- Peak tailing

CONCLUSION

In summary, when validating an analytical test method, there are a variety of factors that should be considered. When developing an analytical method, the necessity of these parameters and the validation as a whole must be carefully weighed. Ensuring the analytical method is robust and scientifically sound could prevent improper conclusions from being drawn during routine product analysis.

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